A STATE RESEMBLING TOLERANCE ARISING IN MICE UNDER THE INFLUENCE OF ANTIGEN OF LYSED ERYTHROCYTES

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A lysate obtained by treating sheep's erythrocytes with distilled water, purified by ultracentrifugation in the cold, was shown to possess weak immunogenicity. Its injection into mice caused a state of hyporeactivity to sheep's erythrocytes (the level of the immune response was depressed to 10-25% of the control). The ability of mouse spleen cells to give an immune response to erythrocytes was undisturbed after adaptive transfer. In the early stages after injection of the lysate the spleen cells of the mice had weak suppressive activity when transplanted into intact animals. Blood serum of mice treated with the lysate possessed weak blocking activity which disappeared after absorption of the serum with sheep's erythrocytes. It is concluded that the hyporeactivity arising in the mice after injection of the lysate is due to the presence of antibodies inhibiting the immune response in the serum.

KEY WORDS: hemolysate; antibodies; inhibition of the immune response.

In 1971, Anderson et al. [4] succeeded in isolating from sheep's erythrocytes (SE) a component deprived of immunogenic properties but capable of inducing a state of specific hyporeactivity to erythrocytes in mice. The material they used consisted of a lysate obtained by treating erythrocytes with distilled water, and purified by ultracentrifugation. Later work showed [5, 6] that the hyporeactivity, which these workers described as immunologic tolerance, was unaccompanied by inactivation of the immunocompetent cells but was due to a serum blocking factor. The nature of this factor is unknown; by its properties it differs both from the original "tolerogen" and from blocking antibodies.

The object of this investigation was to continue the study of the mechanisms of the hyporeactivity arising under the influence of erythrocyte lysate.

EXPERIMENTAL METHOD

The erythrocyte lysate was obtained by the method of Anderson et al [4]. The erythrocytes were lysed with distilled water and centrifuged at 4°C for 200 min at 40,000g. The results were the same whether the supernatant was used freshly prepared or preserved in the frozen state at −20°C.

Experiments were carried out on CBA mice and on $(C57BL/6 \times DBA/2)F_1$ hybrids (BDF_1), in both cases males weighing 20-25 g. The erythrocyte lysate was injected intraperitoneally either in a dose of 0.5 ml on 5 successive days or as a single injection of the total dose (2.5 ml), equivalent to about $1.25 \cdot 10^9$ original erythrocytes.

The reactivity of the animals was determined from the number of 19S-antibody-forming cells (AFC) in the spleen [8] on the 5th day after an intraperitoneal test injection of $2 \cdot 10^8$ SE.

Syngeneic mice, irradiated on the Stebel'-3A apparatus in a dose of 900 R or treated with cyclophosphamide in a dose of 200 mg/kg [1] were used in the adoptive transfer experiments.

The results were subjected to statistical analysis by Student's t-test (geometric mean values and confidence intervals were calculated at the $P \leq 0.05$ level).

EXPERIMENTAL RESULTS

Five injections of the SE lysate or a single injection of the total dose induced weak AFC production in the animals: 440 (372-521) and 776 (550-1094) AFC respectively per spleen (tested 5 days after the course of in-

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TABLE 1. Effect of Single Injection of SE Lysate on Ability of Mice to Give Immune Response

Interval between injection of hemolysate and test injection of SE	Number of AFC in spleen	
	experiment	control
2 days	24300 (14310—41260) $n=4$	45360 (23810—86440) $n=3$
5 days	10850 (7000—16800) $n=4$	
6 days	12750 (6592—31050) $n=4$	45080 (26180—77670) $n=7$
2 months	3643 (2342—5669) $n=5$	28380 (9550—84330) $n=5$

Legend. Here and in Tables 2 and 3, n represents number of mice.

TABLE 2. Crossed Transplantation of Spleen Cells of "Tolerant" (treated with SE lysate) and Normal Mice

Index	Number of AFC in spleen	
	recipients treated with cyclophosphamide	irradiated recipients
Response of spleen cells of tolerant mice after transplantation into normal recipients	4752 (2765—8185) $n=7$	2040 (1441—2888) $n=8$
Response of spleen cells of normal mice after transplantation into normal recipients	4971 (2731—9044) $n=5$	2077 (1436—3002) $n=3$
Response of spleen cells of normal mice after transplantation into tolerant recipients	148 (120—182) $n=5$	294 (105—825) $n=6$

Legend. Spleen cells of tolerant mice used for transplantation 13 days after injection of SE lysate.

TABLE 3. Suppressor Activity of Spleen Cells of Tolerant (treated with SE lysate) Mice

Interval between end of course of injections of SE lysate and transplantation of spleen cells, days	Number of AFC in spleen	
	after transplantation of $50 \cdot 10^6$ spleen cells of tolerant mice + SE	after injection of SE (control)
5	20780 (15330—28160) $n=15$	38160 (30400—49040) $n=13$
14	49700 (32910—75190) $n=7$	35750 (15970—80020) $n=6$

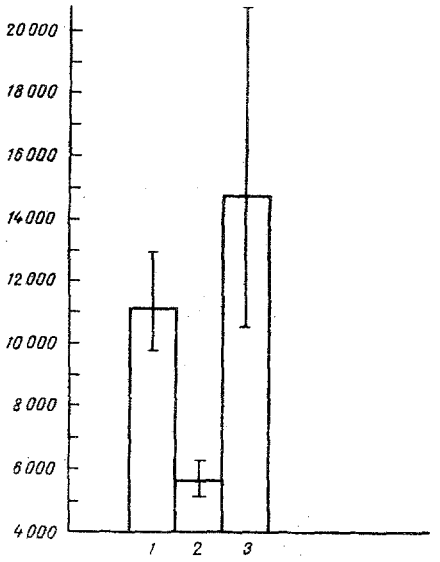

Fig. 1. Suppressor activity of spleen cells of BDF_1 mice receiving SE lysate or native SE. Spleen cells taken on 14th day after injection of lysate or SE and injected in a dose of $70 \cdot 10^6$ into intact syngeneic mice together with $2 \cdot 10^8$ SE. The number of AFC in the recipients' spleen was determined 5 days after transplantation. 1) Recipients receiving SE (control); 2) recipients receiving spleen cells of immune mice+SE; 3) recipients receiving spleen cells of mice treated with lysate+SE. Ordinate, here and in Figs. 2 and 3, number of AFC in spleen.

jections or the single injection of the lysate). The animals' serum contained hemagglutinins (titer 1:80-1:160) and hemolysins (titer 1:20-1:80).

Determination of the number of AFC in response to the test injection of SE given at different times after "tolerogenic" treatment of the mice gave the results shown in Table 1. Clearly a tendency for immunoreactivity to diminish was observed as early as 2 days after injection of the lysate, and 5-6 days after injection the immune response was reduced by three-quarters compared with the control. After 2 months the experimental animals responded as before to the antigen much less strongly (less than one-seventh) than the controls.

In the analogous experiments carried out in the course of the investigation this result was reproduced regularly; the immune response of animals treated with lysate lay between 10 and 25% of the control level (after five injections of lysate or one injection of the total dose).

In the next experiments the reactivity of spleen cells of mice treated with lysate was investigated in adoptive transfer. As Table 2 shows, spleen cells of experimental mice, if transplanted into normal syngeneic recipients (irradiated or treated with cyclophosphamide), gave the same response to SE as spleen cells of control (intact) animals. Conversely, after transplantation of normal spleen cells into irradiated or cyclophosphamide-treated tolerant mice, AFC production was sharply inhibited.

The experiments in which spleen cells of tolerant mice were transplanted into intact mice gave the following results (Table 3). If cells obtained 5 days after the end of the course of injections of lysate were used for transfer, the recipients' immune response was significantly although not considerably depressed (by less than half). Later (14th day) the transplanted cells of tolerant mice did not affect AFC production in the recipients. This result differs from those obtained previously [2], which showed that after immunization of mice

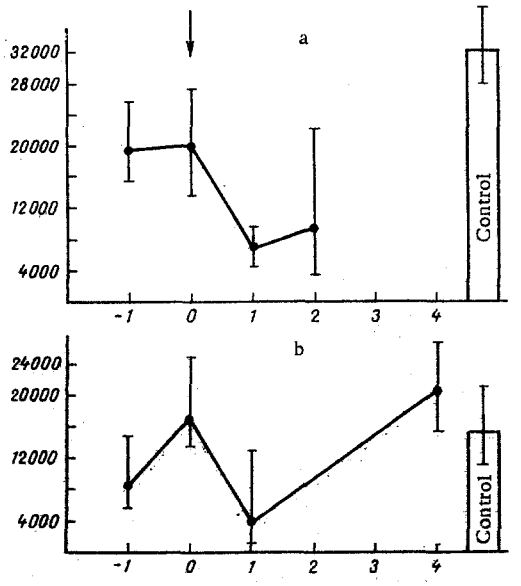

Fig. 2. Effect of serum of mice receiving SE lysate on immune response of intact mice: a) serum of CBA mice taken 5 days after injection of lysate; b) serum of CBA mice taken 12 days after injection of lysate. Abscissa, day of injection of serum relative to day of injection of antigen (arrow).

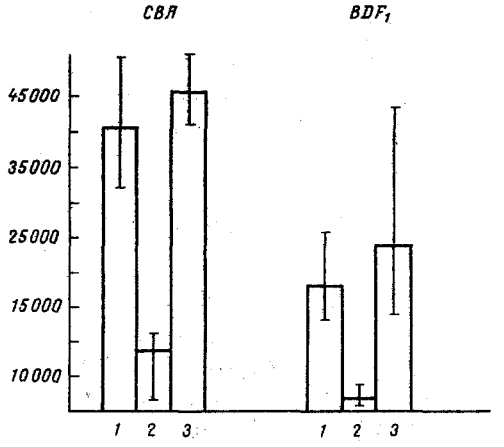

Fig. 3. Effect of absorption of serum of tolerant mice by SE on its inhibitory properties. Serum injected 24 h after injection of SE into mice. 1) Control (no serum injected); 2) serum before absorption; 3) serum after absorption.

with native SE maximal accumulation of suppressor cells was observed toward the 14th day. This difference was demonstrated in the present investigation; also in experiments in which the suppressor activity of spleen cells from mice receiving lysate or native SE was investigated (Fig. 1).

In the last series of experiments the effect of serum of tolerant animals on the immune response of intact mice was studied. Serum was obtained 5 or 12 days after injection of the lysate in a dose of 0.35-0.4 ml (dilution of serum 1:2) and was injected into the mice at different times relative to the time of injection of SE. The results are given in Fig. 2 and they show that the test serum had the power to inhibit AFC production; maximal suppression took place when the serum was injected 24-48 h after the SE. Preliminary triple absorp-

tion of the serum with SE (1 h, 30 min and 30 min at room temperature; ratio of SE to serum 1 : 10 by volume) completely abolished its inhibitory action (Fig. 3).

Comparison of the results of this investigation with those obtained by other workers [4-6] shows that although the erythrocyte lysate used in the present experiments possessed weak antigenicity, the partial areactivity to SE arising after its injection possessed the same features as that described by Auerbach et al. [4, 6]: a reduction in the height of the immune response to 10-25% of the control level, maintenance of hyporeactivity for a relatively long time, absence of inactivation of immunocompetent cells, and the presence of a blocking factor in the blood serum.

By contrast with the results of Auerbach and Roethle [6], the blocking activity of the serum in the present experiments could be completely abolished by absorption with native erythrocytes. Comparison of this fact with the presence of antierythrocytic antibodies in the serum before its absorption suggests that it is these antibodies which perform the function of the blocking factor.

The role of the suppressor cells in the system described above is evidently unimportant. Weak suppressive activity of the spleen cells was found only in the early stages after injection of the SE lysate, and later it could not be detected despite persistence of the state of hyporeactivity.

The state of depressed immunologic reactivity arising in mice after injection of erythrocyte lysate into the animals thus differs from those forms of tolerance to SE that are due either to clonal elimination [3] or to the activity of suppressor cells [7]. The state resembling tolerance described above is evidently a manifestation of a regulatory function of antibodies of feedback type.

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